SYNOPSIS

Inverse Electron Demand Diels Alder Reaction of Pyrido[1,2-a]pyrimidine-2-ones - Experimental and Theoretical Investigation and Antibacterial Activity of Synthesized Adducts

Submitted by

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Submitted to

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September 2022

INTRODUCTION

Heterocyclic ring systems are essential scaffolds for the construction of new drugs. Pyrido[1,2-*a*]pyrimidin-2-ones is one such pharmaceutically interesting class of heterocycles, which has not been much exploited in medicinal chemistry research. But its regioisomer Pyrido[1,2-*a*]pyrimidin-4-ones have been less studied for the their biological properties and abundant synthetic routes are available for the synthesis of its derivatives[1]. Hence the present study aims at the synthesis of fused Pyrido [1, 2-*a*] pyrimidin-2-ones by Inverse electron Demand Diels alder reaction.

Diels-Alder reaction is one of the most powerful synthetic methods applied for the construction of six membered heterocyles. In the normal Diels-Alder reaction electron-donating substituents are present on the diene and electron-withdrawing substituents reside on the dienophile. Attachment of an electron donating group results in the raising of the energy of its frontier molecular orbitals, whereas the presence of an electron-withdrawing group on the dienophile has the effect of lowering the energies of its frontier molecular orbitals (FMOs).

In Inverse Electron Demand Diels Alder reaction (IEDDAR) electron withdrawing groups are attached to the diene and electron donating groups are attached to the dienophile. Similar to the normal Diels-Alder reaction, the complimentary electron rich/electron poor pairing of the reaction partners has the effect of lowering one of the HOMO-LUMO energy differences relative to the neutral reaction [2].

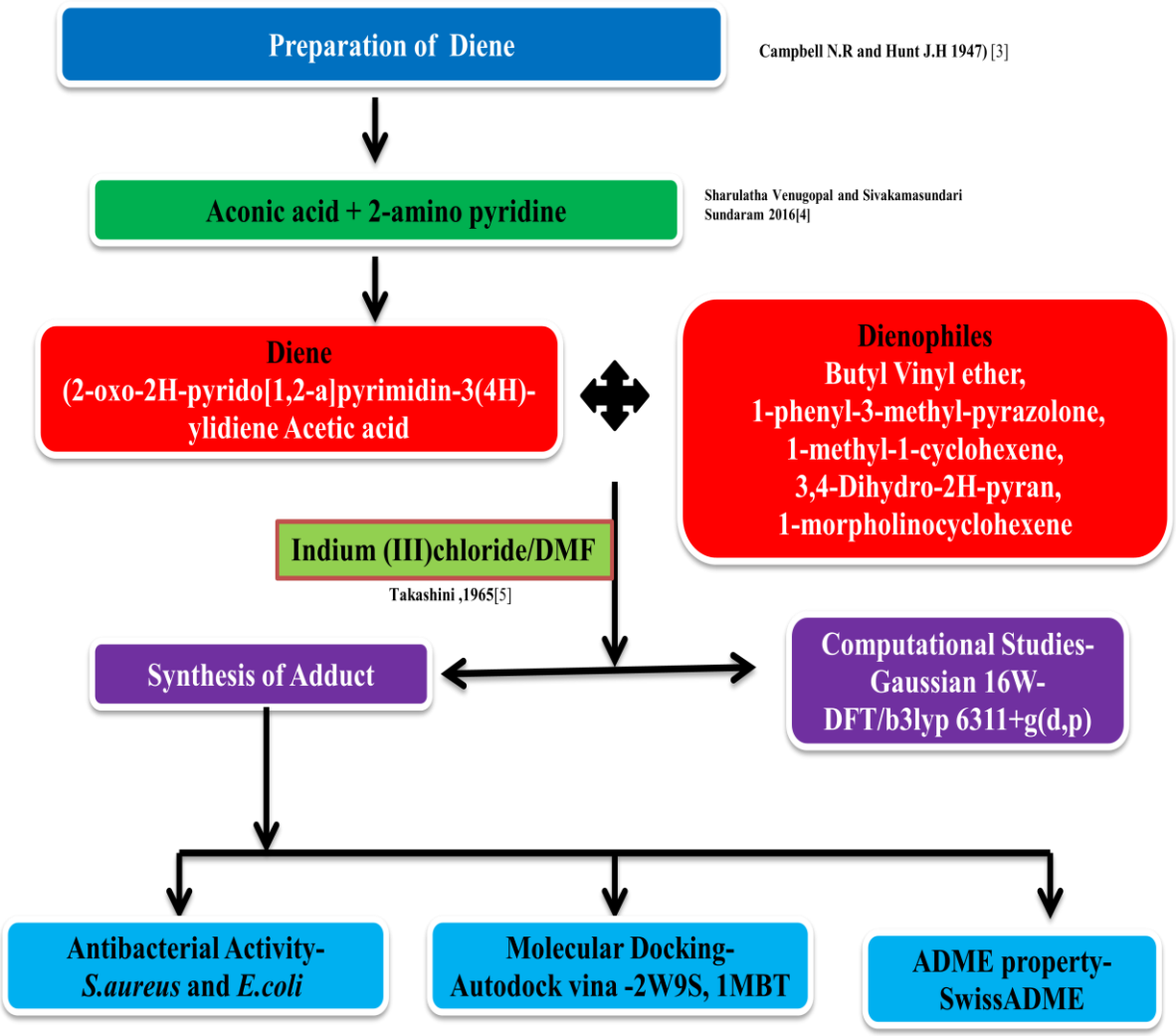
Owing to importance of Pyrido[1,2-*a*]pyrimidin-2-ones ring system we planned to carry out fused Pyrido[1,2-*a*]pyrimidin-2-ones by IEDDAR. In the present study 2-oxo-2H-pyrido[1,2-a]pyrimidin-3(4H)-ylidiene acetic acid act as an electron poor diene and butyl vinyl ether, 1-phenyl-3-methyl-pyrazolone, 1-methyl-1-cyclohexene, 3,4-dihydro-2H-pyran, 1-morpholinocyclohexene act as electron rich dienophiles.

Objective of the study

- ✓ To synthesis fused 2-oxo-2H-pyrido [1, 2-a] pyrimidin-3(4H)-ylidiene acetic acid using indium (III) Chloride as Lewis acid catalyst by IEDDAR with different dienophiles such as butyl vinyl ether, 1-phenyl-3-methyl-pyrazolone, 1-methyl-1-cyclohexene, 3, 4-dihydro-2H-pyran, 1-morpholinocyclohexene.
- ✓ To characterize the compounds with FT-IR, ^{1}H NMR , ^{13}C NMR and Mass Spectra

- ✓ To study the mechanistic details of the IEDDAR by computational method
- ✓ To study the antibacterial activity of the synthesized compound against the gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli.*
- ✓ To carry out insilico studies for the synthesized compounds with the target receptors Staphylococcus aureus (PDB-2W9S) and Escherichia coli (PDB-1MBT).
- ✓ To correlate the invitro studies with the insilico studies.

MATERIALS AND METHOD



RESULTS AND DISCUSSION

Phase –I

Synthesis and characterization of fused 2-oxo-2H-pyrido [1, 2-a] pyrimidin-3(4H)-ylidiene acetic acid

Scheme 1 represents the reaction between 2-oxo-2H-pyrido [1, 2-a] pyrimidin-3(4H)-ylidiene acetic acid and its 7-methyl and 8-methyl substituent's with dienophile butyl vinyl ether in presence of indium chloride catalyst. Melting point and yield of the compounds are given in Table –I. The reaction yielded two isomers *endo* and *exo* in the ratio of 1: 1 as assigned by proton NMR.

Scheme-I

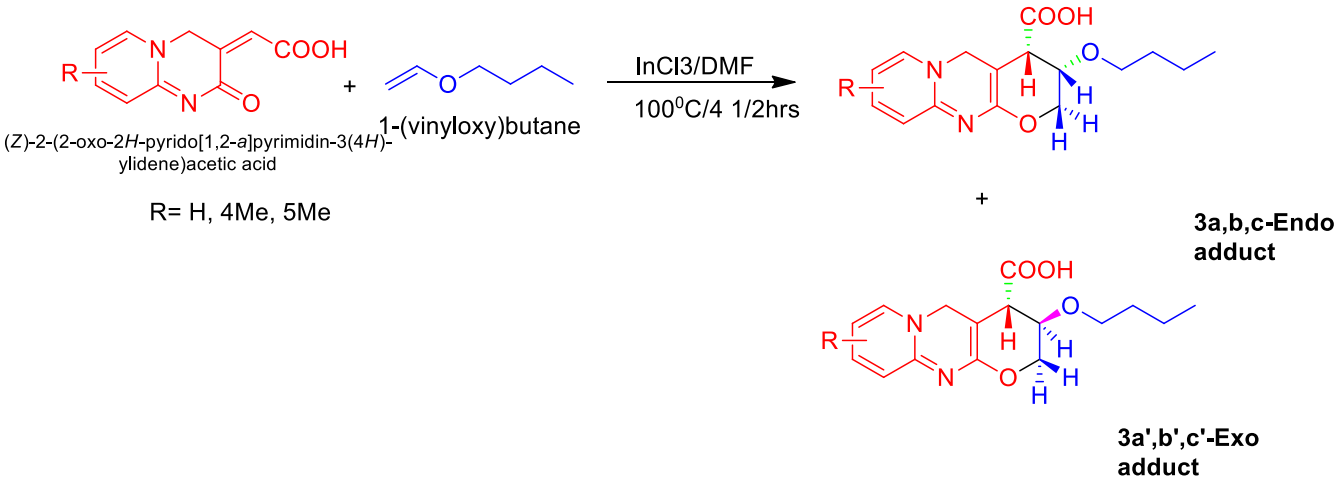


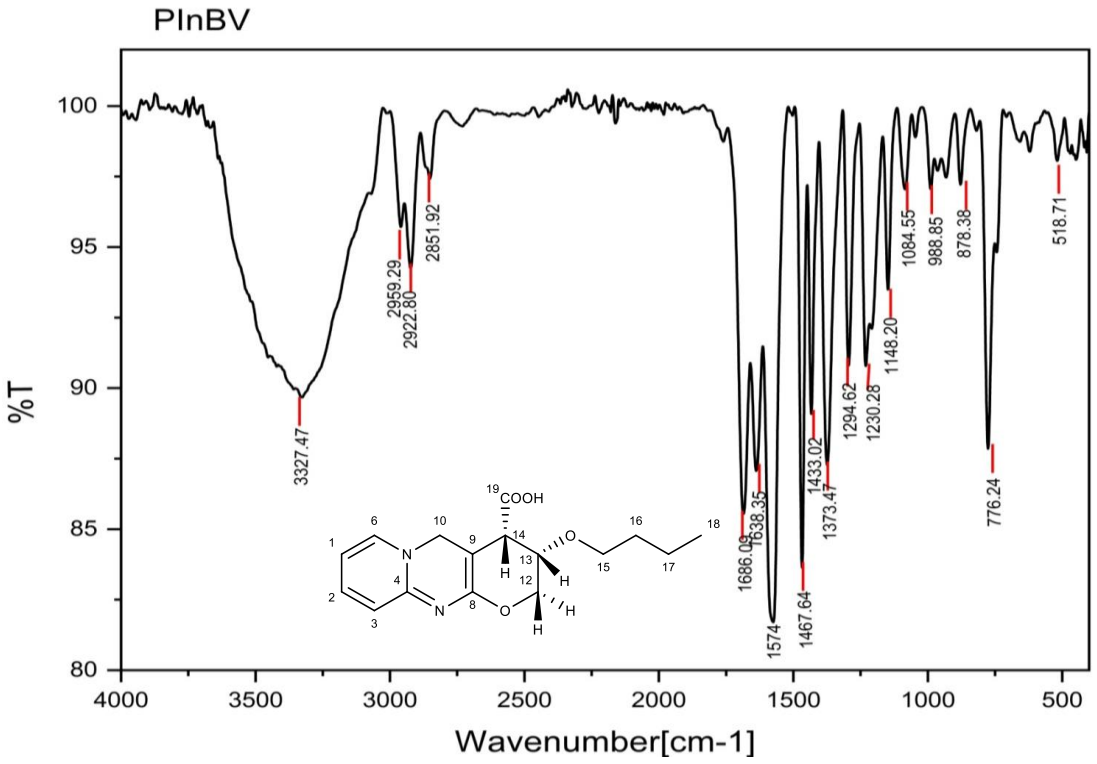
Table-I Melting points and Yield of the compound 3

Diene	Dienophile	Adduct	Catalyst/Solvent/ Temperature	Time / Color	Yield	M.P
2-oxo-2H-pyrido[1,2-a]pyrimidin-3(4H)-ylidiene acetic acid **(1a)**	Butyl vinyl ether **(2)**	**3a**	i)$InCl_3$ /DMF/50˚C ii) $InCl_3$ /DMF/100˚C	i)12 hrs Brown ii) 4 1/2 hrs brown	55%	280-285
(Z)-2-(7-methyl-2-oxo-2H-pyrido[1,2-a]pyrimidin-3(4H)-ylidene)acetic acid**(1b)**		**3b**	i)$InCl_3$ /DMF/100˚C	i) 5 hrs brown	58%	292-297
(Z)-2-(8-methyl-2-oxo-2H-pyrido[1,2-a]pyrimidin-3(4H)-ylidene)acetic acid**(1c)**		**3c**	i)$InCl_3$ /DMF/100˚C	i) 51/2 hrs brown	59%	298-300

FT-IR

The IR spectrum of compound 3a product showed broad intense O-H stretching band centered at 3327 cm-1 which is overriding spectral feature of the carboxylic acid. This was also confirmed by the presence of strong intense O-H in plane bend at 1373 cm^{-1}along with medium intense O-H out of plane bend at 988cm-1. The strong and medium intense bands at 1686 cm-1 and 1294 cm-1 were attributed to the -C=O and -C-O stretch of the carboxylic acid moiety respectively. The absence of strong absorption band at 1680-1700 cm^{-1} confirmed the formation of the product.

Figure: 1 FTIR Spectrum of 3-butoxy-2, 3, 4, 5-tetrahydropyrano [2, 3-d] pyrido [1, 2-a] pyrimidine-4-carboxylic acid (3a)



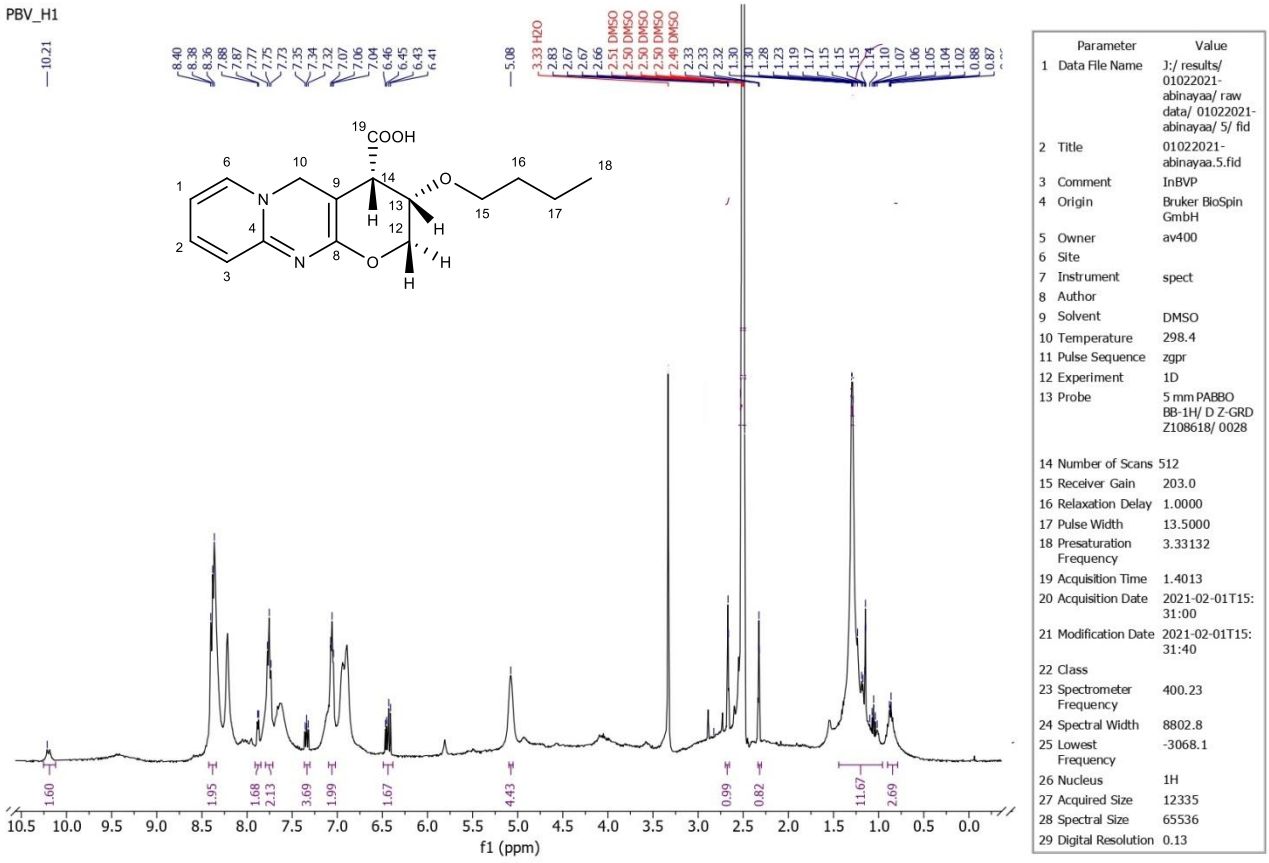
Proton NMR

The proton NMR spectrum displayed eleven sets of protons. 14-H appeared as two triplets with equal intensity, one at δ2.3 and the other at δ2.6, with J value of 4Hz. Hence the formation of *endo* and *exo* isomers in the ratio 1:1 was confirmed [6]. The ring proton 13-H near to oxygen appeared as doublets of doublets at δ6.4-6.46 with J value of 4Hz and 8Hz.

The low coupling constant J=4 Hz observed for 13H with 14-H, against the expected J value of 10Hz for the cis-isomer may be due to the presence of electro negative oxygen atom attached to the C-13 carbon atom [7].

The 12-H appeared as triplet at δ 7.3-7.32 with J value 8Hz and 4Hz. The COOH resonated at δ 10.2, aromatic ring protons appeared at δ 7.05-8.3 and aliphatic protons attached to oxygen atom appeared as multiplet at δ 1.03-1.15 as expected.

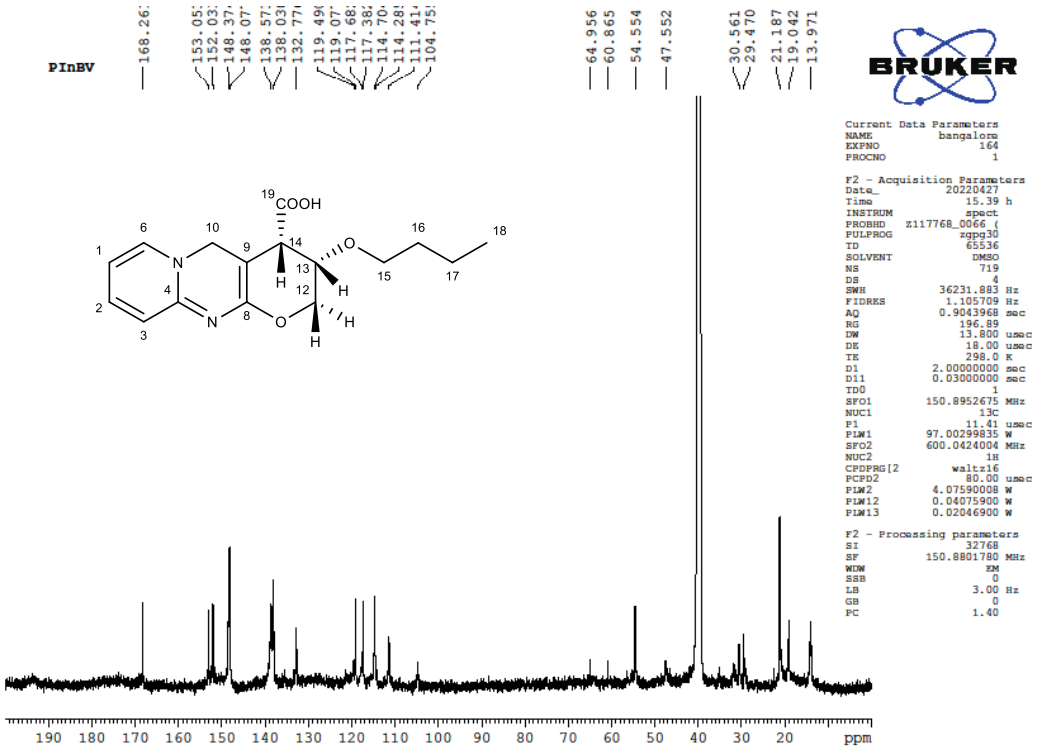
FIGURE-2 ^{1}HNMR Spectrum of 3-butoxy-2, 3, 4, 5-tetrahydropyrano [2, 3-d] pyrido [1, 2-a] pyrimidine-4-carboxylic acid (3a)



^{13}C NMR

The C^{13} NMR spectrum of compound 3a showed sixteen sets of signals. The peak at δ 13 was assigned to the methyl group. C-15, C-16 and -C17 appeared at δ64, 21 and 19 ppm. The peaks at δ 153,152, 148, 138, 132, 119, 117 and 54 were assigned for pyridopyrimidine ring. The carboxylic carbon appeared at δ 168.

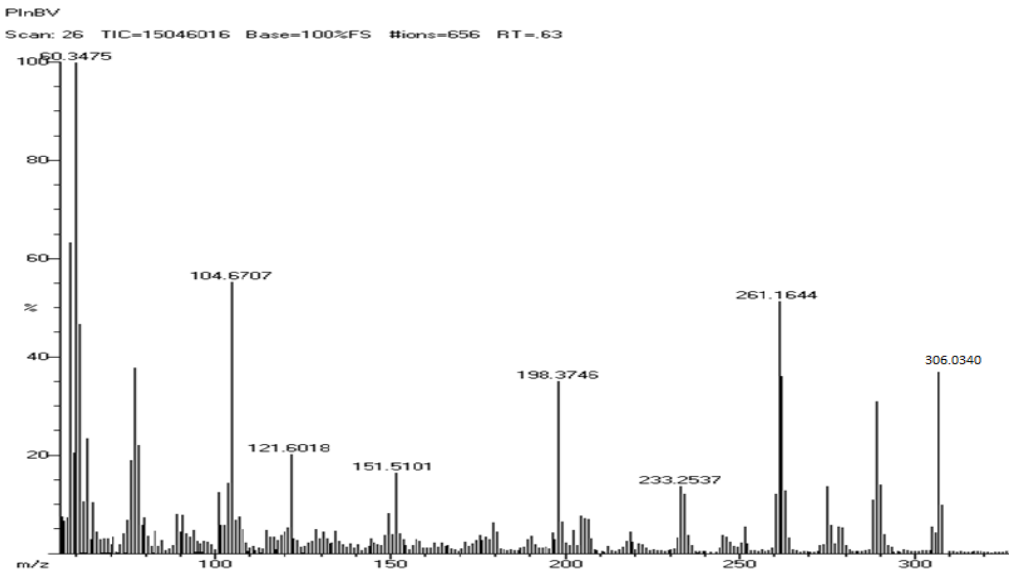
FIGURE-3 ^{13}C Spectrum of 3-butoxy-2, 3, 4, 5-tetrahydropyrano [2, 3-d] pyrido [1, 2-a] pyrimidine-4-carboxylic acid (3a)



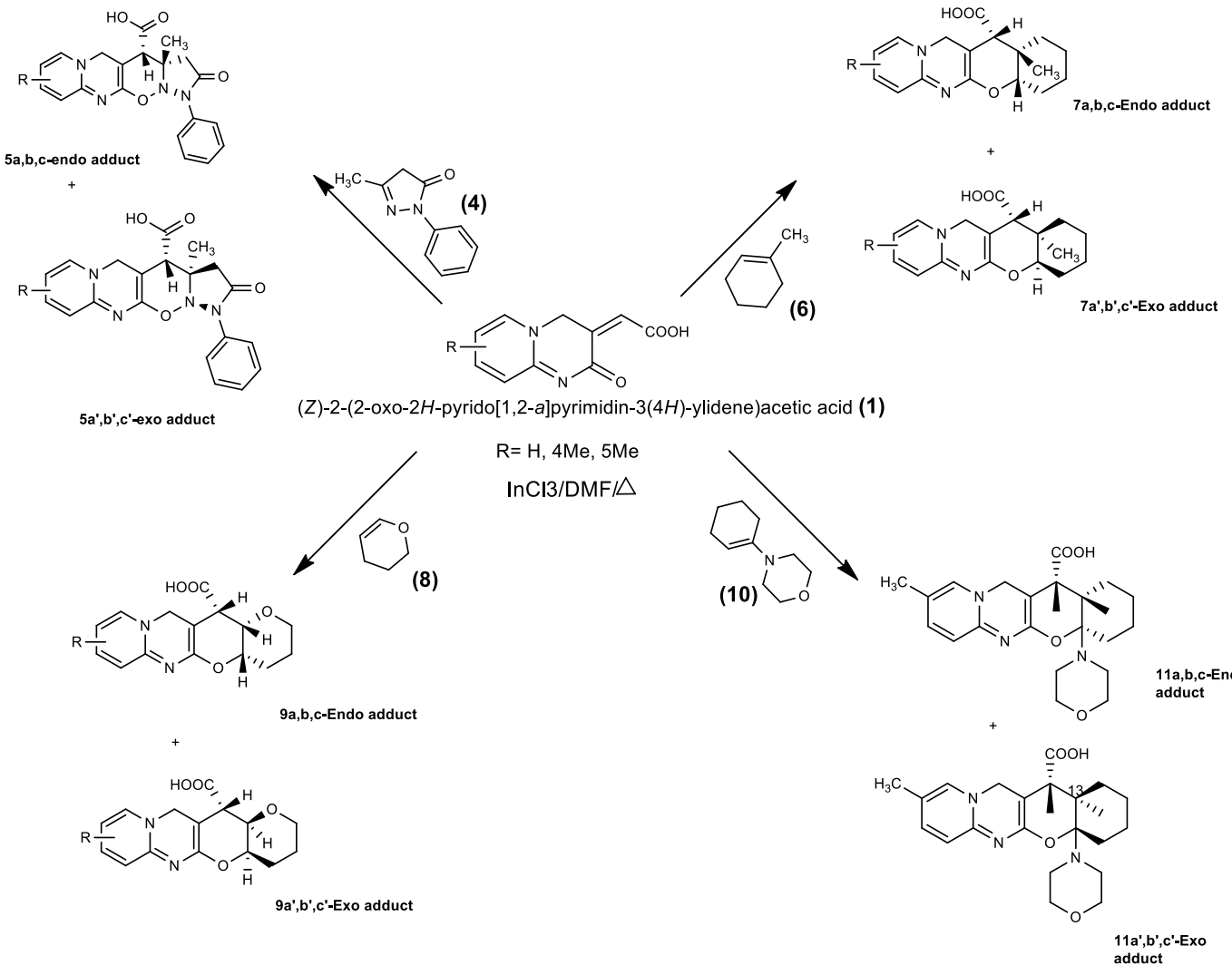
Mass spectra

The calculated m/z ratio of compound 3a was found to be 304.1423 (100.0%). The mass spectrum showed M^{2+} peak at m/z 306.03 in accordance with the proposed structure.

FIGURE-4 Mass Spectrum of 3-butoxy-2, 3, 4, 5-tetrahydropyrano [2, 3-d] pyrido [1, 2-a] pyrimidine-4-carboxylic acid (3a)



Other compounds synthesized using different dielnophiles is represented in Figure -5



Phase –II

Computational studies of fused 2-oxo-2H-pyrido [1, 2-a] pyrimidin-3(4H)-ylidiene acetic acid

To get an insight into the mechanistic pathway of the studied IEDDAR between 2-oxo-2H-pyrido[1,2-a]pyrimidin-3(4H)-ylidiene acetic acid (diene) and various dienophiles computational calculations were performed using Gaussian16W software programme by Density functional theory (DFT)/B3LYP technique with the 6-311+g(d,p) basis set .

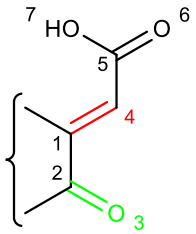
HOMO –LUMO energy gap of Diene and Dienophiles

When the energy gap between LUMO of dienophile and HOMO of diene (**Ldo-Hde**) is lower it favours normal diels alder reaction and when energy gap between LUMO of diene and HOMO of dienophiles (**Lde-Hdo**) is lower it favours inverse electron demand diels alder reaction [8]. The calculated HOMO and LUMO energy gap of diene and dienophiles are give in Table-II. From the table it was observed that the energy gap between LUMO of diene and HOMO of dienophiles (2.45 to 4.25 eV) is lower, indicating the the reaction follows inverse demand diels alder reaction pathway.

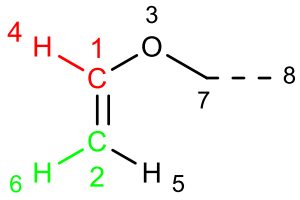
Table-II: Calculated HOMO and LUMO energy gap of diene (1) and dienophiles (2-6)

Diene/dienophile	HOMO (eV)	LUMO (eV)	Ldo-Hde (eV)	Lde-Hdo (eV)
2-oxo-2H-pyrido[1,2-a]pyrimidin-3(4H)-ylidiene Acetic acid (1)	**-6.4028**	**-2.3682**	-	-
Butyl vinyl ether (2)	**-6.2711**	**0.11075**	**6.5136**	**3.9029**
1-phenyl-3-methyl-pyrazolone (4)	**-6.6197**	**-1.0468**	**5.3560**	**4.2515**
1-methyl-1-cyclohexene (6)	**-6.3759**	**0.03020**	**6.4330**	**4.0077**
3,4-dihydro-2H-pyran (8)	**-6.0975**	**0.02803**	**6.43086**	**3.7293**
1-morpholinocyclohexene (10)	**-5.2934**	**-0.07157**	**6.3313**	**2.9252**

NBO and Mulliken charge analysis (Butyl vinyl ether)



DIENE (1)



DIENOPHILE (2)

By the rules of perturbation molecular orbital theory, the cycloaddition will happen in such a way as to unite the atoms with the highest molecular orbital coefficients because this would lead to the greater stabilization [9].

It was observed that the NBO coefficient for C2-terminus of the dieneophile is higher (3.70095) than that for the C1 terminus (2.54623). Similarly, NBO charge of the diene was higher at the O3-terminus (-1.6952) as compared with the C4 terminus (−1.6952). Therefore, the most favourable regioisomeric channel will be that associated with C2–O3 and C1–C4 reaction coordinates, which was in good agreement with experimental outcome. The same trend was observed for the analysis of Mulliken charges. It further confirmed the IEDDAR Pathway.

Transition state and Intrinsic Reaction Coordinate (Butyl vinyl ether)

Comparison of energy barriers of *endo* and *exo* isomers showed less energy for *endo* (-**1032.3638**H) then the other isomers. This is in accordance with *endo* rule which state that in the presence of Lewis-acids, *endo*-products are favoured regioisomers to a higher extent[10] Table-III. IRC path was traced to check the energy profiles connecting each transition state to the associated minima energy of the product/reactant for the proposed mechanism. The intrinsic reaction coordinates showed lower activation energy (-1032.3956 H) for the *endo* transition state and higher activation energy (-1032.40170 H) for the *exo* transition state but the energies of *endo*(-1032.360463 H) and *exo*(-1032.360809 H) products are approximately equal hence the formation of *endo* and *exo* isomer in 1:1 ratio was proved.

Table-III: The energy barriers of *endo* and *exo* transition state

SYSTEM	2-oxo-2H-pyrido [1,2-a]pyrimidin-3(4H)-ylidiene acetic acid(diene)	Butyl vinyl ether	Transition state *endo a*	Transition state *Endo b*	Transition state *Exo a*	Transition state *Exo b*	cycloadducts
Total energy(Hartree)	-721.2377	-311.151	-1032.363	-1032.360	-1032.359	-1032.353	-1032.426

Phase –III

Antibacterial studies of fused 2-oxo-2H-pyrido [1, 2-a] pyrimidin-3(4H)-ylidiene acetic acid

The synthesized compounds were evaluated for their in vitro antibacterial activity against ***Staphylococcus aureus*** **[11]** and ***Escherichia coli*** **[12]** by disc diffusion method against the standard drug **Kanamycin** with different concentration like 20, 40, 60, 80,100µg/ml. Overall compound 5a was found to be more potent against both the bacteria studied with 16mm and 27mm zone of inhibition at 80µg/ml.

Phase –IV

Molecular Docking of fused 2-oxo-2H-pyrido [1, 2-a] pyrimidin-3(4H)-ylidiene acetic acid

Docking studies were carried out for the compounds, for which the activity studies were done. For *Staphylococcus aureus* bacteria the selected receptor protein was Dihydrofolate reductase (DHFR) enzyme (PDB-2W9S)[11] and for *Escherichia coli* bacteria the selected receptor protein was Uridine diphospho-N-Acetylenolpyruvyl Glucosamine reductase enzyme (PDB-1MBT) [12]. The binding energies with the target receptors 2W9S and 1MBT for compounds 5a and 5c was found to be -7.9 and -8.6 Kcal/mol and -8.3 and -8.6 Kcal/mol respectively. This is in accordance with invitro studies. These results indicated that the interactions between our compounds and the target receptor may be effective, indicating that the molecules under study are biologically active.

Phase-V

Pharmacokinetic properties of fused 2-oxo-2H-pyrido [1, 2-a] pyrimidin-3(4H)-ylidiene acetic acid

All of the compounds satisfied Lipinski's rule of five [11] and the values of molecular properties. It means that all the compounds have good binding affinity and theoretically would not have any problem with oral bioavailability.

CONCLUSION

- Fused 2-oxo-2H-pyrido[1,2-a]pyrimidin-3(4H)-ylidiene acetic acid were prepared by IEDDAR with different dienophiles butyl vinyl ether, 1-phenyl-3-methyl-pyrazolone, 1-methyl-1-cyclohexene, 3,4-dihydro-2H-pyran, 1-morpholinocyclohexene using Lewis acid catalyst indium (III) Chloride.

- All the synthesized compounds were characterized and the structures were confirmed using FT-IR, ^{1}H NMR, ^{13}C NMR, and Mass Spectral studies.

- The higher yield (68%) was obtained for the reaction of 2-oxo-2H-pyrido [1, 2-a] pyrimidin-3(4H)-ylidiene acetic acid with 1-phenyl-3-methyl-pyrazolone **(5a)**.

- All the compounds showed diastereo selectivity of 6:1 for endo and exo isomers except for (3s,4s)-3-butoxy-9-methyl-2,3,4,5-tetrahydropyrano[2,3-d]pyrido[1,2-a]pyrimidine-4-carboxylic acid **(3a)** which showed a 1:1 ratio.

- Analysis of FMO, NBO and Mulliken Charge using B3LYP/6-311+G(d,p)basis sets by DFT method proved that the reaction followed the IEDDAR pathway. This was also further confirmed through the transition state energy and intrinsic reaction coordinate (IRC) calculations.

- Synthesized compounds were tested for antibacterial activity and all the compounds exhibited moderate activity and the compound (3as,4s)-3a-methyl-2-oxo-1-phenyl-1,2,3,3a,4,5-hexahydropyrazolo[1,5-b]pyrido[1',2':1,2]pyrimido[5,4-e][1,2]oxazine-4-carboxylic acid **(5a)** was found to be more potent in both insilico and invitro studies.

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